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DRUG METABOLISM AND FOREIGN MATTER HYDROXYLASE (CYTOCHROME P-450) IN SEVERE HEPATIC DAMAGE IN MAN

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16. Abstract In cases of severe hepatic damage (acute hepatitis, active hepatic cirrhosis), a significant reduction is found in the level of cytochrome P 450, in N and O demethylation, and in the activities of pseudocholinesterase and glucose-6-phosphate in human liver biopsy homogenates. Induction of cytochrome P 450, cytochrome c reductase and O demethylation is detected in liver homogenate after the administration of Rifampicin, Chlortritylimidazol or diphenylhydantoin and phenobarbital. The elimination of Rifampicin from the blood is markedly delayed in patients with acute hepatitis or hepatic cirrhosis. Half-life of tolbutamide significantly shortened in acute hepatitis; close correlation in vitro between bilirubin concentration and percentage of free tolbutamide. These findings illustrate the dependence of drug metabolism upon the concentration of microsomal enzyme systems in the liver.

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DRUG METABOLISM AND FOREIGN MATTER HYDROXYLASE (CYTOCHROME P-450) IN SEVERE HEPATIC DAMAGE IN MAN

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. Micromethods developed at the Tübingen Toxikologisches /403* Institut make it possible to determine the level of cytochrome P 450, the rate of demethylation of aminopyrine or p-nitroanisole, and the activities of individual enzymes such as the NADPH-dependent cytochrome c reductase, pseudocholinesterase and glucose-6-phosphatase in homogenates of about 20 mg liver tissue [1]. It thereby becomes feasible to obtain insight into drug metabolism in patients with hepatic damage from whom liver biopsies have been taken for diagnostic reasons.

Drugs and other foreign matter are bound to cytochrome P 450 /404 in the endoplasmic reticulum of the liver cell, as shown in Fig. 1, and can take up molecular oxygen after the reduction of iron in heme, via a specific flavin enzyme system, likewise retained in the endoplasmic reticulum; this results in a mixed functional hydroxylation of foreign aliphatic and aromatic substances. After reduction and exposure to CO, cytochrome is converted into CO-cytochrome, which possesses a characteristic absorption peak at 450 nm, from which the designation cytochrome P 450 is derived. The activity of this foreign matter hydroxylase is a function of species, age, sex and nutritional condition [2, 3, 4]. The enzyme is induced by a large number of foreign substances. When presented in relatively large quantities, various substrates compete for the attachment sites on the protein carrier of the enzyme [5], so substances with high affinity can inhibit the oxidation of other drugs.

* Numbers in the margin indicate pagination in the foreign text.

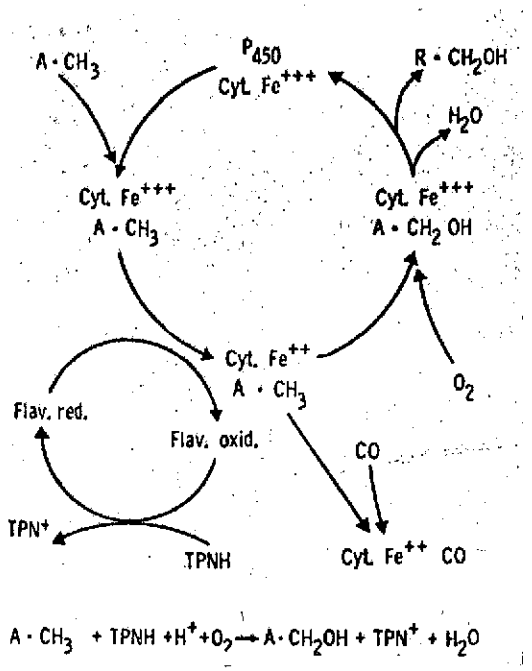

Fig. 1. Diagram of the mixed functional hydroxlyation of drugs (A) by cytochrome P 450 and a specific flavin enzyme system of the liver microsomes (after Reumer).

We have studied the levels of several enzymes which participate in drug metabolism and the in vivo conversion of drugs in liver biopsies from patients with acute hepatitis, alcoholic hepatic damage, and hepatic cirrhosis. A significant drop in the concentration of cytochrome P 450 and a distinct reduction in N and O demethylation was found (Fig. 2) in cases of severe hepatitis or hepatic cirrhosis, whereas patients who, according to biochemical and histological criteria, experienced mild or moderate hepatitis exhibited no appreciable change in drug metabolism. The activities of pseudocholinesterase and glucose-6-phosphatase in the liver were likewise significantly lowered in moderate and severe hepatitis /405 and hepatic cirrhosis. In a 20-year-old woman with serum hepatitis and intrahepatic cholestasia, the level of cytochrome P 450 was at first considerably reduced, but was normalized again in just 4 weeks, in spite of a cholestasia which was still quite pronounced in histological terms. Moreover, four out of 13 habitual drinkers with noncirrhotic alcoholic hepatic damage exhibited a distinct reduction in the activity of cytochrome P 450.

In contrast, considerable induction of cytochrome P 450, and sometimes also of NADPH-cytochrome c reductase and p-nitroanisole O demethylation, can be observed after just brief administration of Chlortritylimidazol (Bayer b 5097), a new oral antimycotic, with combined doses of diphenylhydantoin and phenobarbital (Fig. 3),

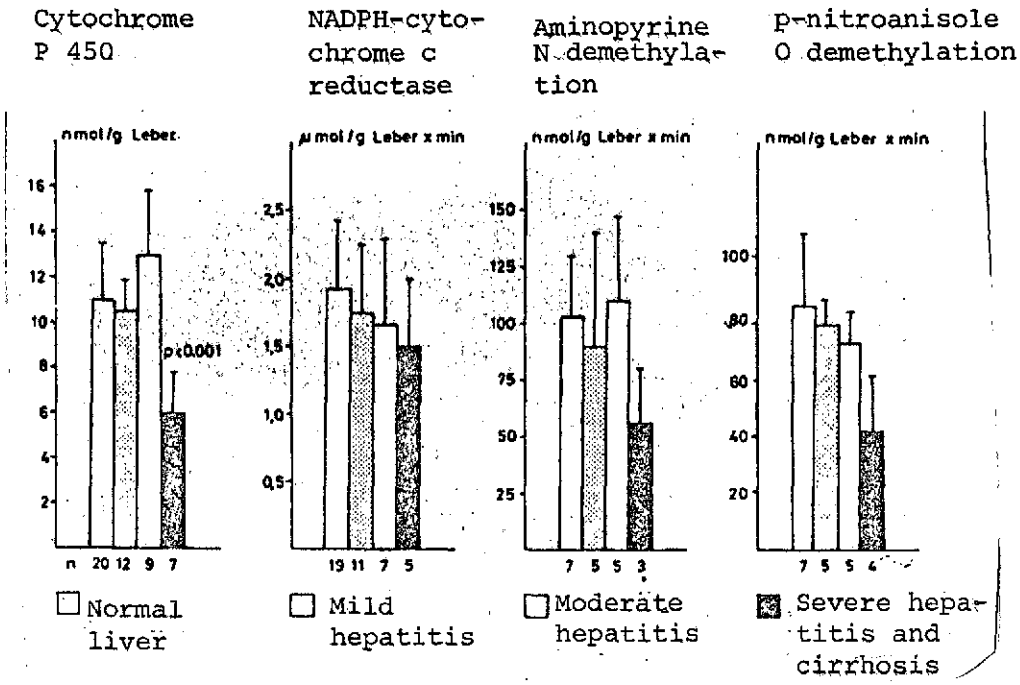

Fig. 2. Levels and activity of microsomal drug-degrading enzymes in liver biopsies from patients with hepatitis or hepatic cirrhosis.

Key: Leber = liver

as well as under a combined tuberculostatic therapy (Fig. 8), in which Rifampicin can cause appreciable induction even by itself. Induction of the drug-degrading hydroxylase can lead to a pronounced acceleration of drug metabolism, so we were no longer able to detect a measurable blood and fluid level when the antimycotic was administered for a long term in a systemic mycosis; this made a 2- or 3-times higher dosage necessary.

The reduction in drug-degrading activities in cases of severe /406 liver damage can result in a delayed elimination of drugs, as illustrated by microbiological studies of the plasma level of Rifampicin in [the] agar- ... [printing error in original; one line apparently missing] ... diffusion disorders after one oral administration of 450 mg Rifampicin. The plasma half-life is increased to 6 hours in hepatic cirrhosis and 6 1/2 hours in acute hepatitis, as opposed to 2 1/2 hours in patients with normal

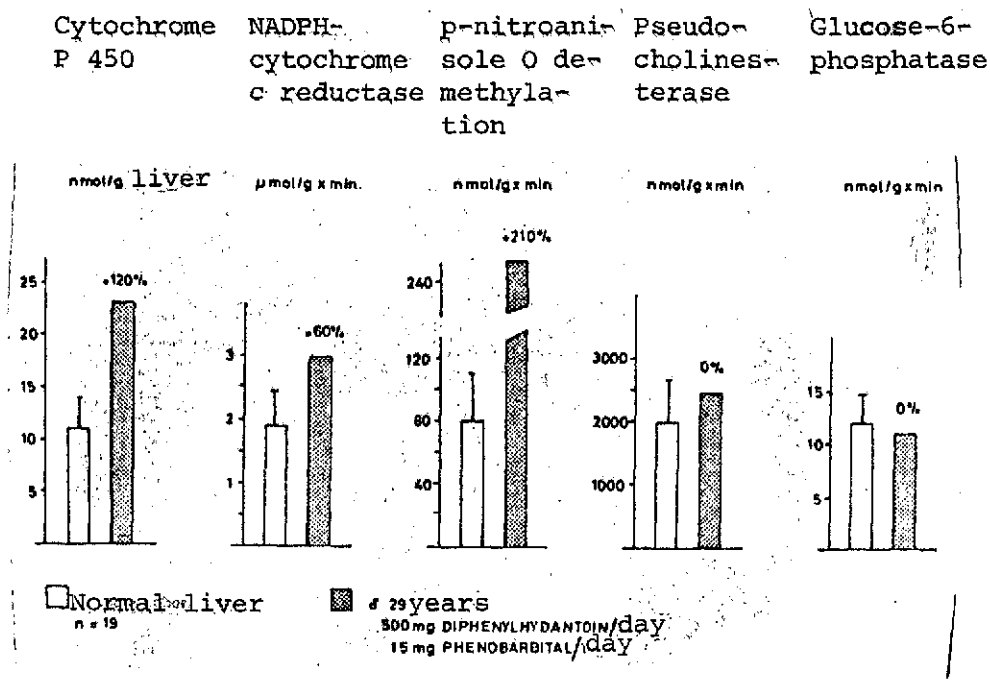

Fig. 3. Levels or activities of microsomal drug-degrading enzymes in the liver of a patient with epilepsy being treated with diphenylhydantoin and phenobarbital.

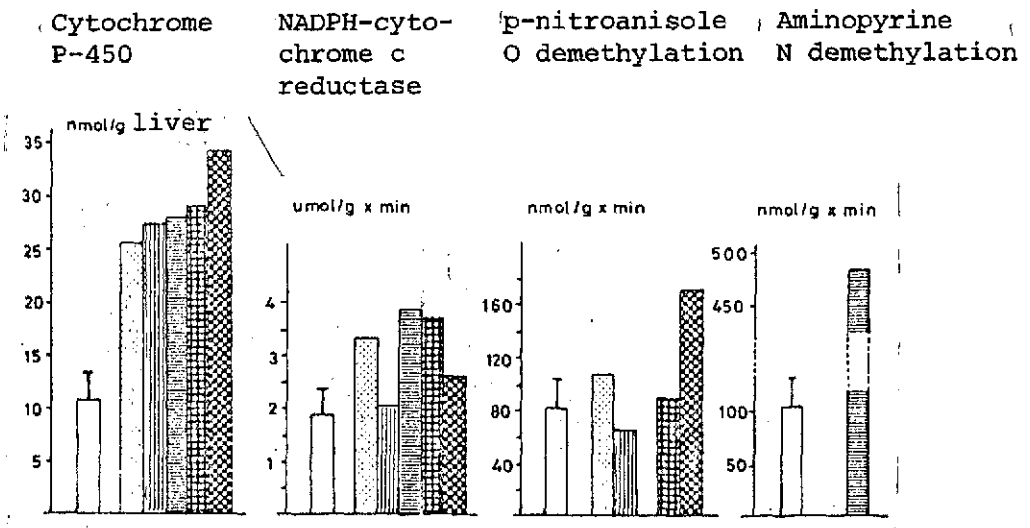

Fig. 4. Combined treatment with INH, streptomycine, ethambutol and Rifampicin.
Untextured column = normal liver
n = 19

livers. On the other hand, the renal elimination of Rifampicin and its microbiologically active metabolites in cases of acute and chronic hepatic damage is hardly altered. This amounts to 25% of the oral dose in 24 hours in subjects with normal livers, 27% in cases of hepatic cirrhosis and 39% in acute hepatitis. As the acute phase of hepatitis subsides, the plasma elimination of Rifampicin becomes normalized [6]. The delayed elimination of Rifampicin in cases of acute hepatitis is apparently attributable to a reduction in hydrolytic enzymes and reduced deacetylation in the liver. Since no correlation can be detected between half-lives and serum bilirubin levels, only limited importance can be ascribed to competitive absorption and excretion mechanisms [7, 8]. Maximum plasma levels are strikingly low and delayed in patients with extrahepatic cholestasia, possibly as the result of disturbed resorption of the Rifampicin (of very low solubility in water) and limited biliary excretion of the metabolies.

In contrast to the behavior of Rifampicin in cases of acute and chronic damage, we observed a significantly accelerated elimination of tolbutamide in cases of hepatitis (Table 1). After a single brief infusion of 12 mg/kg tolbutamide in NaCl followed by glucose infusion for hypoglycemia prophylaxis, all patients with acute or subacute hepatitis exhibited a distinctly reduced half-value time ($\bar{x} \pm s$: 3.47 ± 0.96 hours) relative to subjects with normal livers and kidneys ($\bar{x} \pm s$: 7.12 ± 2.15 hours). After abatement of the acute phase of the disease, the half-lives /408 generally returned to the normal range (Fig. 6). There was no correlation between the level of serum bilirubin and the half-life of tolbutamide.

In order to test whether tolbutamide is displaced from its combination with albumin by elevated bilirubin concentrations in the serum, 100 µg tolbutamide/ml serum was added to the icteric serum of patients with viral hepatitis, hepatic cirrhosis or obstructive jaundice, and the concentration of dialyzable

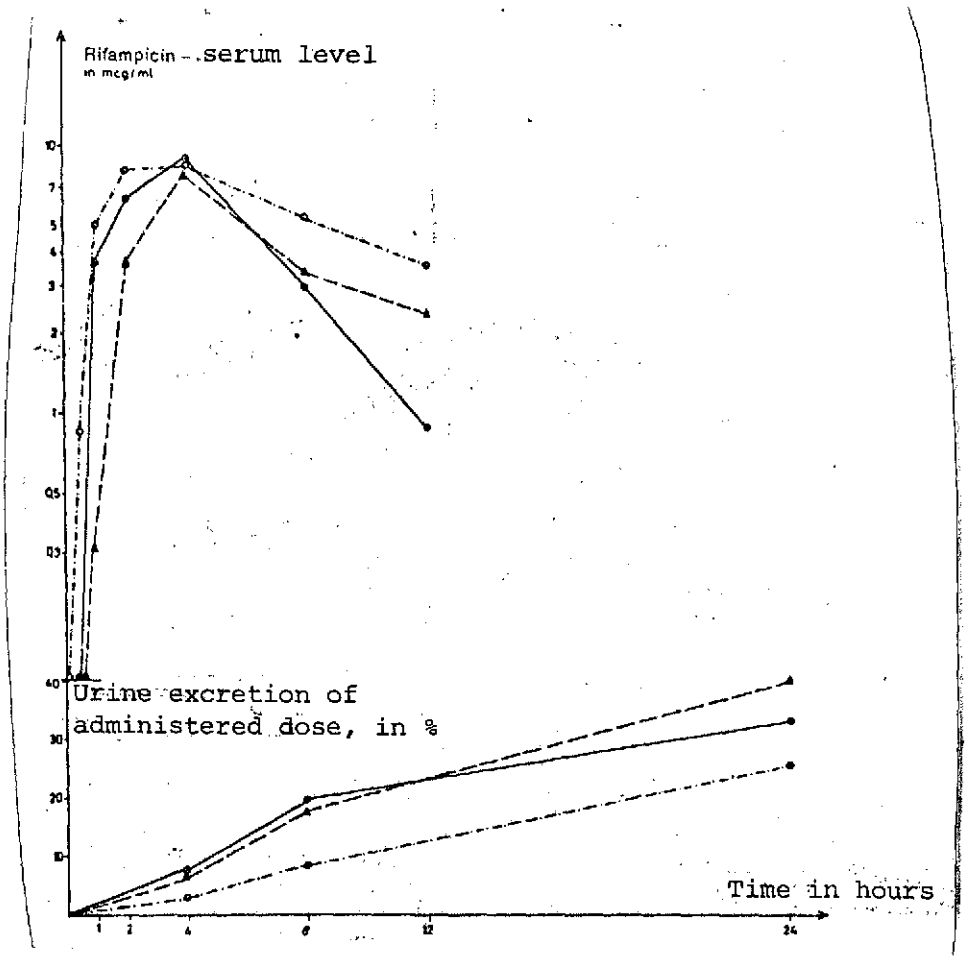

Fig. 5. Rifampicin serum levels in normal subjects, patients with hepatic cirrhosis and with hepatitis during the course of 12 hours, and percentage excretion as Rifampicin in the urine.

● = Normal subjects (n = 7)
o = Patients with hepatic cirrhosis (n = 7)
▲ = Patients with hepatitis (n = 5)

TABLE 1. PLASMA ELIMINATION OF TOLBUTAMIDE. HALF-LIVES AFTER I.V. INJECTION OF 12 mg TOLBUTAMIDE/kg BODY WEIGHT

	N	x ± s	Range of fluctuation
Subjects with normal livers and kidneys	10	7.21 ± 2.15 hr.	5.16 – 11.09 hr.
Patients with viral hepatitis	9	3.47 ± 0.96 hr.	2.24 – 5.00 hr.

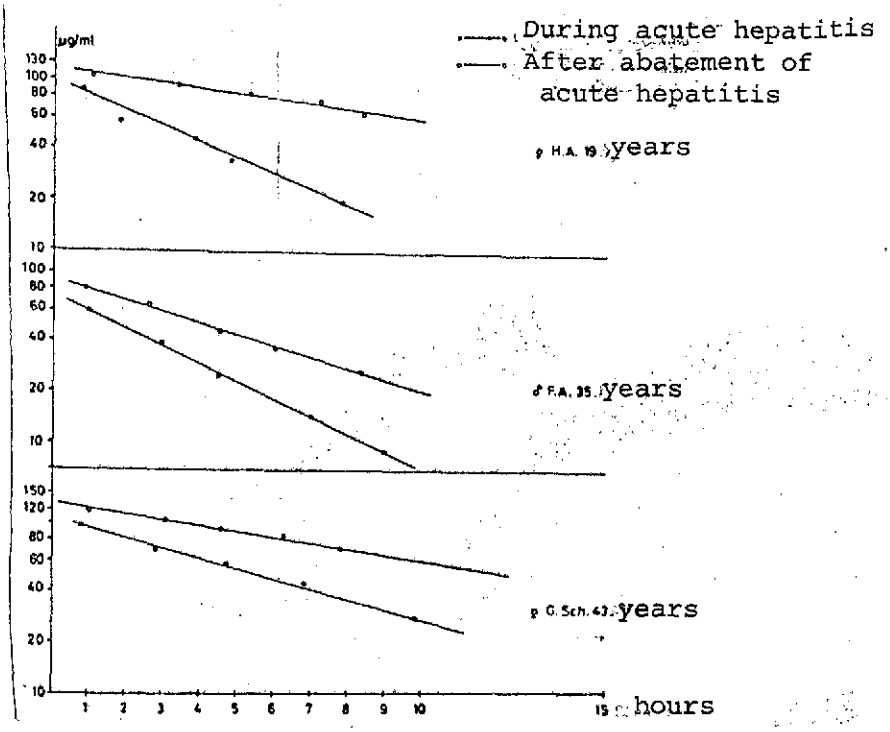

Fig. 6. Elimination of tolbutamide during and after acute hepatitis.

tolbutamide, i.e. not bound to serum albumin, was determined spectrophotometrically after equilibrium dialysis against phosphate buffer for 15 hours. Tolbutamide concentrations (Table 2) in the buffer after dialysis averaged 8.1 ± 3.8 µg in icteric sera as opposed to 2.7 ± 0.6 µg tolbutamide/ml buffer in the sera of subjects with normal livers ($p < 0.001$). A close correlation was found between the percentage of dialyzable tolbutamide and the serum bilirubin concentration ($r = 0.79$; $p < 0.001$). One patient with hepatic cirrhosis should be mentioned, who exhibited the highest percentage of dialyzable tolbutamide, 27%, with a very low serum albumin level (1.8 g%). In studies of curves before and after the occurrence of serum hepatitis, the percentage of free tolbutamide in the serum rose from 2.0 to 7.9%. Due to displacement of the tolbutamide from its bonding site on serum albumine by bilirubin, the concentration of free, pharmacologically effective tolbutamide is thus sometimes elevated in icteric /409

patients; this could explain the occurrence of hypoglycemias in icteric patients after even small doses of tolbutamide. On the other hand, the more abundantly liberated tolbutamide can be eliminated more rapidly from the blood in cases of hepatitis and be metabolized in the liver. The danger of hypoglycemia in tolbutamide-stabilized diabetics who contract a viral hepatitis is thus limited by the rapid elimination of tolbutamide. It is nevertheless advisable to distribute the daily dose and make blood sugar checks more frequently. Spontaneous, even protracted hypoglycemias can be expected especially with the simultaneous administration of sulfonylureas such as tolbutamide or glycodiazine and phenylbutazone, Dicumarol or doxycycline [9, 10], sometimes attributed to inhibition in NADPH-dependent microsomal enzymes.

TABLE 2. CONCENTRATION OF FREE TOLBUTAMIDE, NOT BOUND TO SERUM ALBUMIN, AFTER THE ADMINISTRATION OF 100 µg TOLBUTAMIDE/ /ml SERUM AND EQUILIBRIUM DIALYSIS VERSUS PHOSPHATE BUFFER.

	N	$\bar{x} \pm s$	Range of fluctuation
Subjects with normal livers	13	2.7 ± 0.6 µg/ml	2.0 – 3.3 µg/ml
Patients with severe icterus	10	8.1 ± 3.8 µg/ml	3.8 – 17.2 µg/ml

These experimental results seem to indicate that the following, among other things, can be considered responsible for the occurrence of undesirable side effects by drugs or failures in drug therapy [10]:

1. A reduction in drug-metabolizing enzyme activities in cases of severe hepatic cell damage, explaining a delayed elimination of drugs in cases of severe viral hepatitis, alcoholic hepatic damage or hepatic cirrhosis which has until now been demonstrated only occasionally,

2. Induction of the microsomal enzyme systems of the liver by previously or simultaneously administered foreign substances such as drugs or perhaps alcohol,

3. A competitive inhibition, by bilirubin, of the binding of drugs to albumin.

Appreciable individual and genetic differences make it necessary to give separate consideration to each individual drug.

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